MycoKeys 82: 33–56 (2021)
doi: 10.3897/mycokeys.82.63241
https://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
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Four new species in the *Tremella fibulifera* complex (Tremellales, Basidiomycota)

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Academic editor: Thorsten Lumbsch | Received 17 January 2021 | Accepted 12 July 2021 | Published 4 August 2021

Citation: Fan L-F, Alvarenga RLM, Gibertoni TB, Wu F , Dai Y-C (2021) Four new species in the *Tremella fibulifera* complex (Tremellales, Basidiomycota). MycoKeys 82: 33–56. https://doi.org/10.3897/mycokeys.82.63241

Abstract

Samples of species close to *Tremella fibulifera* from China and Brazil are studied, and *T. fibulifera* is confirmed as a species complex including nine species. Five known species (*T. cheejenii*, *T. fibulifera* s.s., *T. "neofibulifera"*, *T. lloydiae-candidae* and *T. olens*) and four new species (*T. australe*, *T. guangxiensis*, *T. latispora* and *T. subfibulifera*) in the complex are recognized based on morphological characteristics, molecular evidence, and geographic distribution. Sequences of eight species of the complex were included in the phylogenetic analyses because *T. olens* lacks molecular data. The phylogenetic analyses were performed by a combined sequence dataset of the internal transcribed spacer (ITS) and the partial nuclear large subunit rDNA (nLSU), and a combined sequence dataset of the ITS, partial nLSU, the small subunit mitochondrial rRNA gene (mtSSU), the translation elongation factor 1-α (TEF1), the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2). The eight species formed eight independent lineages with robust support in phylogenies based on both datasets. Illustrated description of the six species including *Tremella fibulifera* s.s., *T. "neofibulifera"* and four new species, and discussions with their related species, are provided. A table of the comparison of the important characteristics of nine species in the *T. fibulifera* complex and a key to the whitish species in *Tremella* s.s. are provided.

Keywords

Multi-gene, phylogeny, taxonomy, Tremellaceae

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Introduction

Tremella Pers. is characterized by being parasitic on or associated with other fungi or lichens (Bandoni and Oberwinkler 1983; Chen 1998; Pippola and Kotiranta 2008; Malysheva et al. 2015; Zhao et al. 2019) and by having a haploid unicellular yeast stage and diploid stage in its life cycle (Boekhout et al. 2011; Weiss et al. 2014; Liu et al. 2015a; Zhao et al. 2019). *Tremella* s.l. includes approximately 90 species and about 50 are recognized as lichenicolous species (Kobayasi 1939; Bandoni 1958, 1987; Lowy 1971; Bandoni and Oberwinkler 1983; Diederich 1996; Chen 1998; Kirk et al. 2008; Pippola and Kotiranta 2008; Millanes et al. 2011, 2012, 2014, 2015, 2016; Malysheva et al. 2015; Zamora et al. 2016; Zhao et al. 2019). *Tremella* s.l. is polyphyletic and was divided into five groups by Chen (1998) including Mesenterica group, Fuciformis group, Indecorata group, Foliacea group and Aurantia group based on morphological characteristics and molecular data of ITS rDNA and nLSU rDNA sequencing. Then species in Mesenterica group and Fuciformis group were allocated to *Tremella* s.s., and species in Indecorata group, Foliacea group and Aurantia group were emended as *Pseudotremella* Xin Zhan Liu et al., *Phaeotremella* Rea and *Naematelia* Fr., respectively (Liu et al. 2015b; Wedin et al. 2016; Spirin et al. 2018). Besides, *Tremella* s.l. contained lichenicolous species that defined as *Tremella* clade I, clade II, clade III, and several single *Tremella* species lineages based on rDNA sequences (Millanes et al. 2011; Liu et al. 2015a, b).

Although *Tremella* s.l. was separated into several genera due to its polyphyletism, it is still somewhat confusing because taxonomic positions of some *Tremella* species are uncertain in Tremellales, especially some species recently described from lichens (Ariyawansa et al. 2015; Malysheva et al. 2015; Millanes et al. 2015; Zamora et al. 2016, 2018). These lichenicolous species were described as *Tremella*, but they were not clustered into *Tremella* s.s. in the phylogeny (Ariyawansa et al. 2015; Malysheva et al. 2015; Millanes et al. 2015; Zamora et al. 2016, 2018). Recently, Zhao et al. (2019) described four new *Tremella* species based on the phylogenetic relationship of 19 species in *Tremella* s.s., and Li et al. (2020) published a new yeast species of *Tremella* s.s. based on multi-gene analysis.

In this study, samples of species morphologically similar to *Tremella fibulifera* characterized by cerebriform whitish basidioma and abundant clamp complexes from China and Brazil are studied. Based on morphology, geographic distribution and phylogenetic analyses *T. fibulifera* is confirmed as a species complex, which was previously mentioned by Bandoni and Oberwinkler (1983) and Malysheva et al. (2015), and nine species are involved in the complex including five known species (*T. cheejenii*, *T. fibulifera* s.s., *T. "neofibulifera"*, *T. lloydiae-candidae* and *T. olens*) and four new species (*T. australe*, *T. guangxiensis*, *T. latispora* and *T. subfibulifera*) in the present study. The aim of this paper is to outline the *T. fibulifera* complex and describe two known species (*T. fibulifera* s.s., *T. "neofibulifera"*) and the four new species based on our collections.

Materials and methods

Sampling and morphological analysis

The studied specimens were collected from Rondônia and Pernambuco states in Brazil, Yunnan, Taiwan, Guangxi, Jilin Provinces in China. They are deposited at the herbaria of Beijing Forestry University (**BJFC**), Institute of Botânica in São Paulo (**SP**) and Universidade Federal de Pernambuco, Departamento de Micologia (**URM**). Macro-morphological illustrations refer to Chen (1998) and Zamora et al. (2017) and microscopic structures refer to Pippola and Kotiranta (2008) and Malysheva et al. (2015). Special color terms followed Petersen (1996). Handmade sections of dried basidioma were examined by a Nikon Eclipse 80i (Japan) microscope (magnification × 1000) after being mounted in 5% KOH for five minutes and treated with 1% Phloxine B ($C_{20}H_4Br_4Cl_2K_2O_5$). Microscopic structures were photographed using a Nikon Digital Sight DS-L3 (Japan) or Leica ICC50 HD (Japan) camera. Microscopic structures were examined and measured in the mix solution of 5% KOH and 1% Phloxine B. To represent variation in the size of spores, 5% of measurements were excluded from each end of the range, and are given in parentheses. Stalks were excluded for basidia measurements and apices were excluded for basidiospores measurements. Length and width of at least 30 basidia and basidiospores from each specimen were measured to micrometers.

Abbreviations as follows: **L** = mean length (arithmetic average of all basidia or spores length), **W** = mean width (arithmetic average of all basidia or spores width), **Q** = L/W ratio for each specimen studied, **n (a/b)** = number of spores (a) measured from given number of specimens (b).

Molecular phylogeny

Dry specimens were used to extract DNA after pretreatment using TissuePrep (Jie Ling, China) by CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) or directly using the DNA easy Plant Mini Kit (Qiagen, China), according to the manufacturer's instructions with some modifications. The internal transcribed spacer regions (**ITS**), partial nuclear large subunit rDNA (nLSU), the translation elongation factor 1-α (TEF1), the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2), the small subunit mitochondrial rRNA gene (mtSSU) sequences were amplified with primer pairs listed in the Table 1. All newly generated sequences were submitted to GenBank (Table 2).

Polymerase chain reaction (**PCR**) cycling schedule for ITS, mtSSU and TEF1 included an initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 40 s, 54–56 °C (ITS) and 56–58 °C (mtSSU, TEF1) for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min, for RPB1 and RPB2 included an initial denaturation at 95 °C for 3 min, followed by 9 cycles at 94 °C for 45 s or 1 min , 58 °C for

Table 1. Sequencing primers used in this study.

Pairs of primer	Nucleotide sequence (5'–3')	Reference
ITS		
ITS5	5'–GGAAGTAAAAGTCGTAACAAGG–3'	White et al. 1990
ITS4	3'–TCCTCCGCTTATTGATATGC–5'	
ITS1	5'–TCCGTAGGTGAACCTGCGG–3'	
Partial nLSU		
LR0R	5'–ACCCGCTGAACTTAAGC–3'	Hopple and Vilgalys 1994
LR7	5'–TACTACCACCAAGATCT–3'	
TEF1		
983F	5'–GCYCCYGGHCAYCGTGAYTTYAT–3'	Rehner 2001
1567R	3'–ACHGTRCCRATACCACCRATCTT–5'	Rehner 2001
2218R	3'–ATGACACCRACRGCRACRGTYTG–5'	Rehner and Buckley 2005
RPB1		
Af	5'–GARTGYCCDGGDCAYTTYGG–3'	Stiller and Hall 1997
Cr	3'–CCNGCDATNTCRTTRTCCATRTA–5'	Matheny et al. 2002
RPB2		
5F	5'–GAYGAYMGWGATCAYTTYGG–3'	Matheny 2005
6F	5'–TGGGGKWTGGTYTGYCCTGC–3'	Matheny 2005
7R	3'–CCCATWGCYTGCTTMCCCAT–5'	Matheny 2005
7CR	3'–CCCATRGCTTGYTTRCCCAT–5'	Matheny 2005
Fcrypto	5'–TGGGGYATGGTTTGTCCKGC–3'	Ye et al. 2012
Rcrypto	3'–CCCATGGCTTGTTTRCCCATYGC–5'	Ye et al. 2012
mtSSU		
MS1	5'–CAGCAGTCAAGAATATTAGTCAATG–3'	White et al. 1990
MS2	3'–GCGGATTATCGAATTAAATAAC–5'	White et al. 1990

45 s or 60 °C for 1 min and 72 °C for 1.5 min, then followed by 35 cycles at 95 °C for 1 min, 53 °C or 55 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min, for partial nLSU included an initial denaturation at 94 °C for 1 min, followed by 34 cycles at 94 °C for 30 s, 50–52 °C for 1 min, 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. PCR products were purified at the Beijing Genomics Institute (BGI), China or at the Plataforma Tecnológica de Genômica e Expressão Gênica do Centro de Biociências (UFPE) with the same primers.

Newly generated sequences in this study were aligned with additional related sequences downloaded from GenBank (Table 2) using MAFFT 7.0 online service with the Q-INS-i strategy (Katoh et al. 2019, http://mafft.cbrc.jp/alignment/server/). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps were manually adjusted to optimize the alignment using the default parameters in BioEdit (Hall 1999). Those positions deemed ambiguous to align were excluded manually. Multi-genes were concatenated as a combined file by Mesquite version 3.2. (Maddison and Maddison 2017). Phylogenetic analyses were applied to the ITS + partial nLSU dataset and the combined ITS+partial nLSU+mtSSU+TEF1+RPB1+RPB2 dataset. Sequences of *Cryptococcus depauperatus* (Petch) Boekhoutet et al. were used as outgroup, which referred to Malysheva et al. (2015). The final concatenated sequence alignments were deposited in TreeBase (https://treebase.org/treebase-web/home.html; submission ID 28280 for ITS + partial nLSU

Table 2. Information on sequences used in this study.

Species	Sample no.	GenBank accessions						References
		ITS	Partial nLSU	mtSSU	TEF1	RPB1	RPB2	
***Tremella australis* sp. nov.**	**Dai 11539**	**MT445847**	–	–	**MT445759**	–	–	**Present study**
***T. australis* sp. nov.**	**Wu 154**	**MT445848**	**MT425188**	**MT483749**	**MT445760**	–	**MT445753**	**Present study**
T. basidiomaticola	CBS 8225	MH712822	MH712786	–	–	–	–	Zhao et al. 2019
T. basidiomaticola	CGMCC 2.5724^{T}	MH712820	MH712784	–	–	–	–	Zhao et al. 2019
T. basidiomaticola	CGMCC 2.5725	MH712821	MH712785	–	–	–	–	Zhao et al. 2019
T. brasiliensis	CBS 6966^{R}	AF444429	AF189864	KF036694	KF037200	–	KF036938	Liu et al. 2015a
T. brasiliensis	CBS 8212	KY105674	KY109886	–	–	–	–	Vu et al. 2016
T. cerebriformis	ZRL 20170101	MH712823	MH712787	–	–	–	–	Zhao et al. 2019
T. cerebriformis	ZRL 20170269	MH712824	MH712788	–	–	–	–	Zhao et al. 2019
T. cheejenii	GX 20172598	MH712825	MH712789	–	–	–	–	Zhao et al. 2019
T. cheejenii	GX 20172640	MH712826	MH712790	–	–	–	–	Zhao et al. 2019
T. dysenterica	LE 303447	KP986509	KP986542	–	–	–	–	Malysheva et al. 2015
T. dysenterica	VLA M 18599	KP986531	–	–	–	–	–	Malysheva et al. 2015
T. erythrina	HMAS 255317	MH712827	MH712791	–	–	–	–	Zhao et al. 2019
T. erythrina	HMAS 279591	MH712828	MH712792	–	–	–	–	Zhao et al. 2019
***T. fibulifera* s.s.**	**SP 211759**	**MT445850**	**MT425190**	**MT483750**	–	–	–	**Present study**
***T. fibulifera* s.s.**	**Alvarenga 471**	**MT445851**	**MT425191**	–	–	–	–	**Present study**
T. flava	CBS 8471^{R}	KY105681	KY105681	KF036699	KF037205	KF036527	KF036943	Liu et al. 2015a
T. flava	CCJ 907	AF042403	AF042221	–	–	–	–	Zhao et al. 2019
T. fuciformis	CBS 6970^{R}	KY105683	AF075476	KF036701	KF037207	KF036529	–	Liu et al. 2015a
T. fuciformis	CCJ1080	AF042410	AF042228	–	–	–	–	Malysheva et al. 2015
T. globispora	CBS 6972^{R}	AF444432	AF189869	KF036703	KF037208	KF036531	KF036947	Liu et al. 2015a
T. globispora	UBC 586	AF042425	AF042243	–	–	–	–	Zhao et al. 2019
***T. guangxiensis* sp. nov.**	**Wu 3**	**MT445843**	**MT425186**	**MT483748**	**MT445756**	**MT445746**	**MT445752**	**Present study**
***T. guangxiensis* sp. nov.**	GX 20172028	MH712829	MH712793	–	–	–	–	Zhao et al. 2019
***T. latispora* sp. nov.**	**Dai 17574**	**MT445852**	**MT425192**	**MT483751**	**MT445761**	**MT445750**	**MT445754**	**Present study**
***T. latispora* sp. nov.**	**Dai 17568**	**MT445853**	**MT425193**	**MT483752**	**MT445762**	**MT445751**	**MT445755**	**Present study**
T. laurisilvae	S-F 102408(AM4)	JN053467	JN043572	–	–	–	–	Zhao et al. 2019
T. lloydiae-candidae	VLA M 11702	KP986536	KP986559	–	–	–	–	Malysheva et al. 2015
T. lloydiae-candidae	VLA M 11703	KP986559	KP986560	–	–	–	–	Malysheva et al. 2015
T. mesenterica	CBS 6973^{R}	AF444433	AF444433	KF036705	KF037210	KF036533	KF036949	Liu et al. 2015a
T. mesenterica	FO 24610	AF042447	AF042265	–	–	–	–	Zhao et al. 2019
T. "neofibulifera"	**Wu 248**	**MT445844**	**MT425187**	–	**MT445757**	**MT445747**	–	**Present study**
T. "neofibulifera"	**Wu 243**	**MT445845**	–	–	–	**MT445748**	–	**Present study**
T. "neofibulifera"	**Wu 244**	**MT445846**	–	–	**MT445758**	**MT445749**	–	**Present study**
T. "neofibulifera"	LE 303445	KP986518	KP986547	–	–	–	–	Malysheva et al. 2015
T. resupinata	CBS 8488^{T}	AF042421	AF042239	KF036708	KF037212	KF036535	KF036951	Liu et al. 2015a
T. saccharicola	DMKU-SP23^{T}	AB915385	AB909021	–	–	–	–	Khunnamwong et al. 2019
T. saccharicola	DMKU-SP40	AB915386	AB909022	–	–	–	–	Khunnamwong et al. 2019
T. salmonea	GX 20172637	MH712851	MH712815	–	–	–	–	Zhao et al. 2019
T. samoensis	LE 303465	KP986508	KP986541	–	–	–	–	Malysheva et al. 2015
T. samoensis	VLA M 18603	KP986532	KP986555	–	–	–	–	Malysheva et al. 2015
T. shuangheensis	CBS 15561	MK050285	MK050285	MK050285	MK849087	MK849223	MK849362	Li et al. 2020

Species	Sample no.	GenBank accessions						References
		ITS	Partial nLSU	mtSSU	TEF1	RPB1	RPB2	
***T. subfibulifera* sp. nov.**	**Alvarenga 334**	**MT445849**	**MT425189**	–	–	–	–	**Present study**
T. taiwanensis	CBS 8479[R]	AF042412	AF042230	KF036709	KF037213	KF036536	KF036952	Liu et al. 2015a
T. taiwanensis	GX 20170625	MH712854	MH712818	–	–	–	–	Zhao et al. 2019
T. tropica	CBS 8483[R]	KY105697	KY109908	KF036710	KF037214	KF036537	KF036953	Liu et al. 2015a
T. tropica	CBS 8486	KY105696	KY109909	–	–	–	–	Liu et al. 2015a
T. yokohamensis	JCM 16989	HM222926	HM222927	–	–	–	–	Zhao et al. 2019
T. yokohamensis	CBS 11776	KY105698	KY109910	–	–	–	–	Malysheva et al. 2015
Cryptococcus depauperatus	CBS 7841[T]	FJ534881	FJ534911	AJ568017	KF037150	KF036471	–	Zhao et al. 2019

The samples used in this study are in bold.

dataset; submission ID 27553 for ITS + partial nLSU + mtSSU + TEF1 + RPB1 + RPB2 dataset) and the taxonomic novelties in MycoBank (http://www.MycoBank.org).

Phylogenetic constructions of Maximum likelihood (ML), Maximum parsimony (MP), and Bayesian analyses were performed in the CIPRES Science Gateway portal Version 3.3 (Miller et al. 2012) using tool of RAxML-HPC BlackBox 8.2.6, PAUP on XSEDE (4.a165) and Mrbayes on XSEDE 3.2.6 respectively. All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using heuristic search option with TBR branch swapping and 1000 random sequence additions. MrModeltest 2.3 (Posada and Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for both datasets for Bayesian analyses using MrBayes3.1.2 (Ronquist and Huelsenbeck 2003). Four Markov chains were run for two runs from random starting trees for 3 million generations (ITS + nLSU) and for 5 million generations (ITS + partial nLSU + mtSSU + TEF1 + RPB1 + RPB2) until the split deviation frequency value < 0.01, and trees were sampled every 100 generation. The first quarter generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated.

Phylogenetic trees were viewed by FigTree v. 1.4.2 (Rambaut 2012) and edited by Adobe Illustrator CS6 (Guide 2012). Branches that received bootstrap support for Maximum parsimony (BP), Maximum likelihood (BS) and Bayesian posterior probabilities (BPP) greater than or equal to 50% (BP/BS) and 0.95 (BPP) were considered as significantly supported, respectively.

Results

Phylogeny

The ITS + partial nLSU dataset included 50 fungal specimens representing 27 species. The dataset has an aligned length of 2282 total characters including gaps, of which 1777 characters are constant, 128 variable characters are parsimony-uninformative, and 377 are parsimony-informative. MP analysis yielded four equally parsimonious trees (TL = 1394, CI = 0.529, RI = 0.792, RC = 0.419, HI = 0.471). The best model

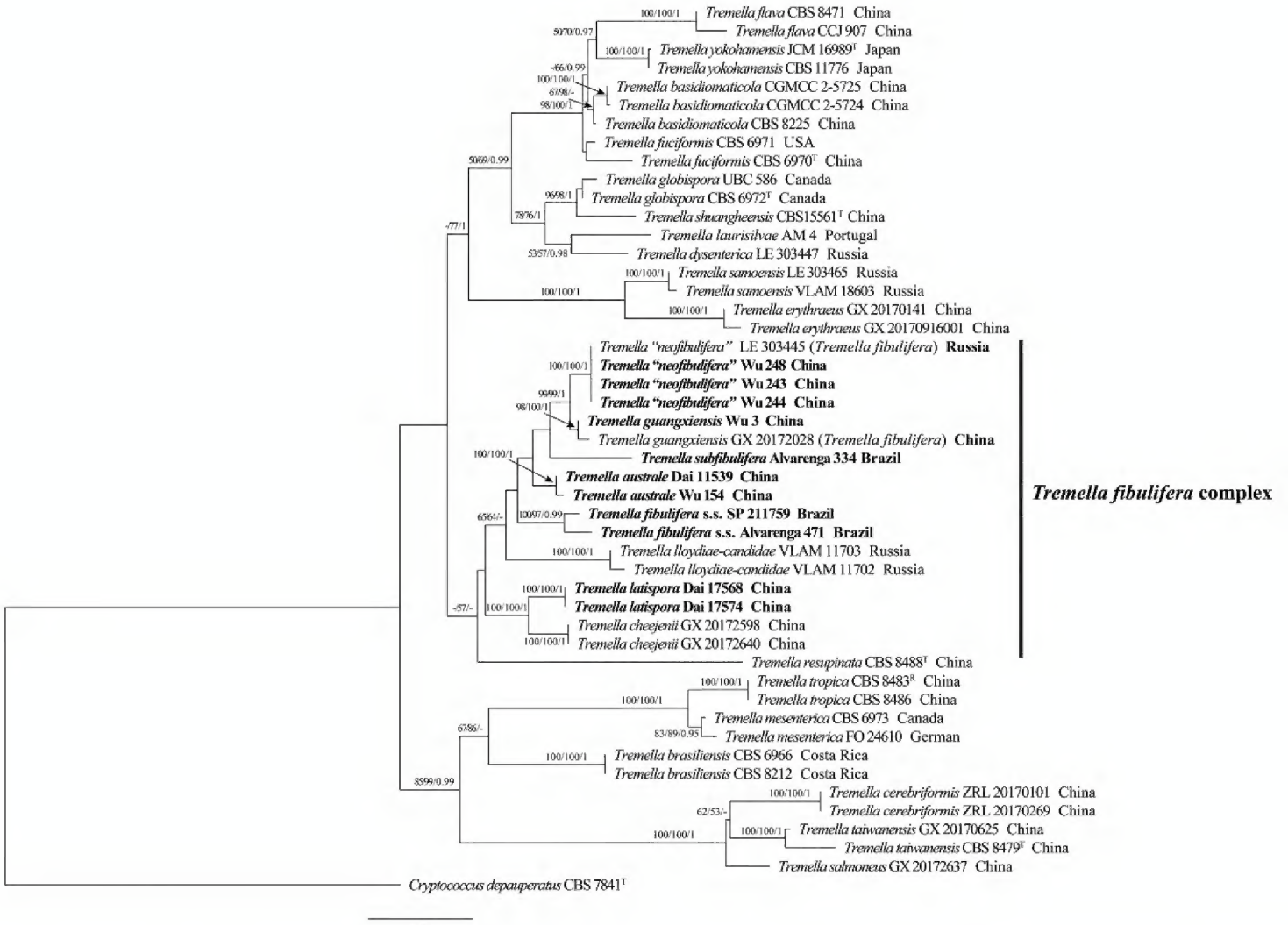
Tremella flava CBS 8471 China
Tremella flava CCJ 907 China
Tremella yokohamensis JCM 16989T Japan
Tremella yokohamensis CBS 11776 Japan
Tremella basidiomaticola CGMCC 2-5725 China
Tremella basidiomaticola CGMCC 2-5724 China
Tremella basidiomaticola CBS 8225 China
Tremella fuciformis CBS 6971 USA
Tremella fuciformis CBS 6970T China
Tremella globispora UBC 586 Canada
Tremella globispora CBS 6972T Canada
Tremella shuangheensis CBS15561T China
Tremella laurisilvae AM 4 Portugal
Tremella dysenterica LE 303447 Russia
Tremella samoensis LE 303465 Russia
Tremella samoensis VLAM 18603 Russia
Tremella erythraeus GX 20170141 China
Tremella erythraeus GX 20170916001 China
Tremella "neofibulifera" LE 303445 (Tremella fibulifera) Russia
Tremella "neofibulifera" Wu 248 China
Tremella "neofibulifera" Wu 243 China
Tremella "neofibulifera" Wu 244 China
Tremella guangxiensis Wu 3 China
Tremella guangxiensis GX 20172028 (Tremella fibulifera) China
Tremella subfibulifera Alvarenga 334 Brazil
Tremella australe Dai 11539 China
Tremella australe Wu 154 China
Tremella fibulifera s.s. SP 211759 Brazil
Tremella fibulifera s.s. Alvarenga 471 Brazil
Tremella lloydiae-candidae VLAM 11703 Russia
Tremella lloydiae-candidae VLAM 11702 Russia
Tremella latispora Dai 17568 China
Tremella latispora Dai 17574 China
Tremella cheejenii GX 20172598 China
Tremella cheejenii GX 20172640 China
Tremella resupinata CBS 8488T China
Tremella tropica CBS 8483R China
Tremella tropica CBS 8486 China
Tremella mesenterica CBS 6973 Canada
Tremella mesenterica FO 24610 German
Tremella brasiliensis CBS 6966 Costa Rica
Tremella brasiliensis CBS 8212 Costa Rica
Tremella cerebriformis ZRL 20170101 China
Tremella cerebriformis ZRL 20170269 China
Tremella taiwanensis GX 20170625 China
Tremella taiwanensis CBS 8479T China
Tremella salmoneus GX 20172637 China
Cryptococcus depauperatus CBS 7841T
Tremella fibulifera complex
0.05

Figure 1. The Maximum likelihood tree showing phylogenetic relationship of species in *Tremella* s.s. based on the ITS + partial nLSU dataset. Bootstrap support values for MP and ML greater than 50% and BI greater than 0.95 are given at each node respectively. The samples used in this study are in bold.

for the ITS + partial nLSU dataset estimated and applied in the BI analysis was GTR. BI and ML analyses generated similar topologies as MP analysis, with an average standard deviation of split frequencies = 0.002648 (BI). The best tree obtained from the ML analysis with bootstrap values for BP, BS and BPP is shown in Fig. 1. The phylogeny shows that eight species are clustered into the *T. fibulifera* complex and four new species form four independent lineages with robust support.

The combined dataset of ITS + partial nLSU + mtSSU + TEF1 + RPB1 + RPB2 has an aligned length of 5113 total characters including gaps, of which 3332 characters are constant, 383 variable characters are parsimony-uninformative, and 1398 are parsimony-informative. MP analysis yielded two equally parsimonious trees (TL = 4519, CI = 0.607, RI = 0.730, RC = 0.443, HI = 0.393). The best model for the combined dataset estimated and applied in the BI analysis was GTR+I+G. BI analysis generated similar topology to MP and ML analysis, with an average standard deviation of split frequencies = 0.008566 (BI). The best tree obtained from the ML analysis with bootstrap values for BP, BS and BPP is shown in Fig. 2. The phylogeny results in similar topology to the phylogeny based on the ITS + partial nLSU sequences, which supports four new species separated from *T. fibulifera* s.s. and *T. "neofibulifera"*.

Taxonomy

Tremella fibulifera Möller, Botanische Mittheilungen aus den Tropen 8: 170 (1895)

Figs 3A, 4

Basidioma. Sessile, when fresh gelatinous, pale whitish, lobed to irregularly cerebriform, becoming pale yellowish when dry, 0.5–2.5 cm in diameter, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, thin- to thick-walled, 2.0–5.0 μm in diameter, branched, interwoven, with abundant clamp connections and medallion clamp connections (clamp complexes), thick-walled hyphae usually present near to base of basidioma; hyphidia hyaline, smooth, thin-walled, branched; swollen cells, vesicles and haustoria absent; mature basidia thin-walled, globose to subglobose, with a basal clamp connection, 13.0–18.0(–22.0) × 9.0–16.0 μm, L = 15.7 μm, W = 14.8 μm, Q = 1.06 (n = 30/1), sometimes their width greater than length, usually longitudinally septate, rarely obliquely septate, 2–4-celled, with obvious oil drops; sterigmata up to 100 μm long, 1.5–2.0 μm in diameter, slightly protuberant at apex; probasidia thin-walled, subglobose to ellipsoid, mostly proliferating directly from basidial clamps; basidiospores hyaline, thin-walled, mostly ellipsoid to slightly ovoid, apiculate, with oil drops, 7.0–10.0 × 6.0–7.0 μm, L = 8.4 μm, W = 6.5 μm, Q = 1.29–1.40 (n = 60/2), germinating by germ tubes or secondary spores; conidia occasionally present in cluster, originating from conidiophores, hyaline, thin-walled, ellipsoid to subglobose, 2.0–3.0 × 1.0–2.5 μm.

Specimens examined. Brazil Rondônia, Municipality of Jaru, in mixed forest near the airport, 9°40'S, 61°50'W, on wood, associated with old pyrenomycete stromata and litter, 10 October 1986, M. Capelari & R. Maziero 944 (SP211759, duplicate BJFC028110); Pernambuco, Recife, Jardim Botânico do Recife, on angiosperm wood, 16 May 2017, R. L. M. Alvarenga 471 (URM).

Notes. *Tremella fibulifera* was probably a species complex including *T. olens* originally from Australia and *T. neofibulifera* originally from Japan because they shared cerebriform whitish basidioma and abundant clamp complexes (Möller 1895; Bandoni and Oberwinkler 1983; Malysheva et al. 2015). Two specimens (SP211759, Alvarenga

Table 3. A morphological comparison of taxa in the *Tremella fibulifera* complex.

Taxa	Basidia (μm)	Basidiospores (μm)	Conidia (μm)	Hyphidia	Distribution	Reference
T. fibulifera	12.0–16.0	7.0–10.0	3.5	Unknown	Brazil	Möller 1895
T. fibulifera	15.0–18.0 × 9.0–13.0	8.0–9.0 × 5.0–8.0	Not observed	Unknown	Brazil	Bandoni and Oberwinkler 1983
T. fibulifera s.s.	13.0–18.0 × 9.0–16.0	7.0–10.0 × 6.0–7.0	2.0–3.0 × 1.0–2.5	Branched	Brazil	Present study
T. australe	14.0–19.0 × 13.0–17.0	8.0–10.0 × 6–8.0	Absent	Present	China	Present study
T. cheejenii	12.0–17.0 × 13.0–18.0	5.0–10.0 × 4.5–8.0	2.2–4.0 × 1.8–3.0	Branched	China	Zhao et al. 2019
T. guangxiensis	14.0–17.0 × 14.0–16.0	8.0–9.5 × 6.0–7.5	2.0–3.2 × 1.8–3.0	Branched	China	Present study
T. latispora	17.2–24.0 × 17.0–23.0	10.1–11.8 × 9.9–11.4	2.8–3.6 × 1.8–3.0	Present	China	Present study
T. lloydiae-candidae	14.0–20.0 × 13.0–16.0	7.5–10	Absent	Unknown	Japan, Russia	Malysheva et al. 2015
T. olens	Unknown	12.7–14.5	Absent	Unknown	Australia	Hooker 1860
T. neofibulifera	13.2–15.5 × 9–10	5.5–8.5 × 4.5–5.5	Absent	Unknown	Japan	Kobayasi 1939
T. "neofibulifera"	14.0–16.0 × 13.0–17.0	8.0–10.0 × 6.0–8.0	Absent	Parallel	China, Russia	Present study
T. subfibulifera	14.4–20.3 × 12.8–16.3	5.4–9.8 × 4.2–6.0	2.0–3.0 × 0.5–1.0	Absent	Brazil	Present study

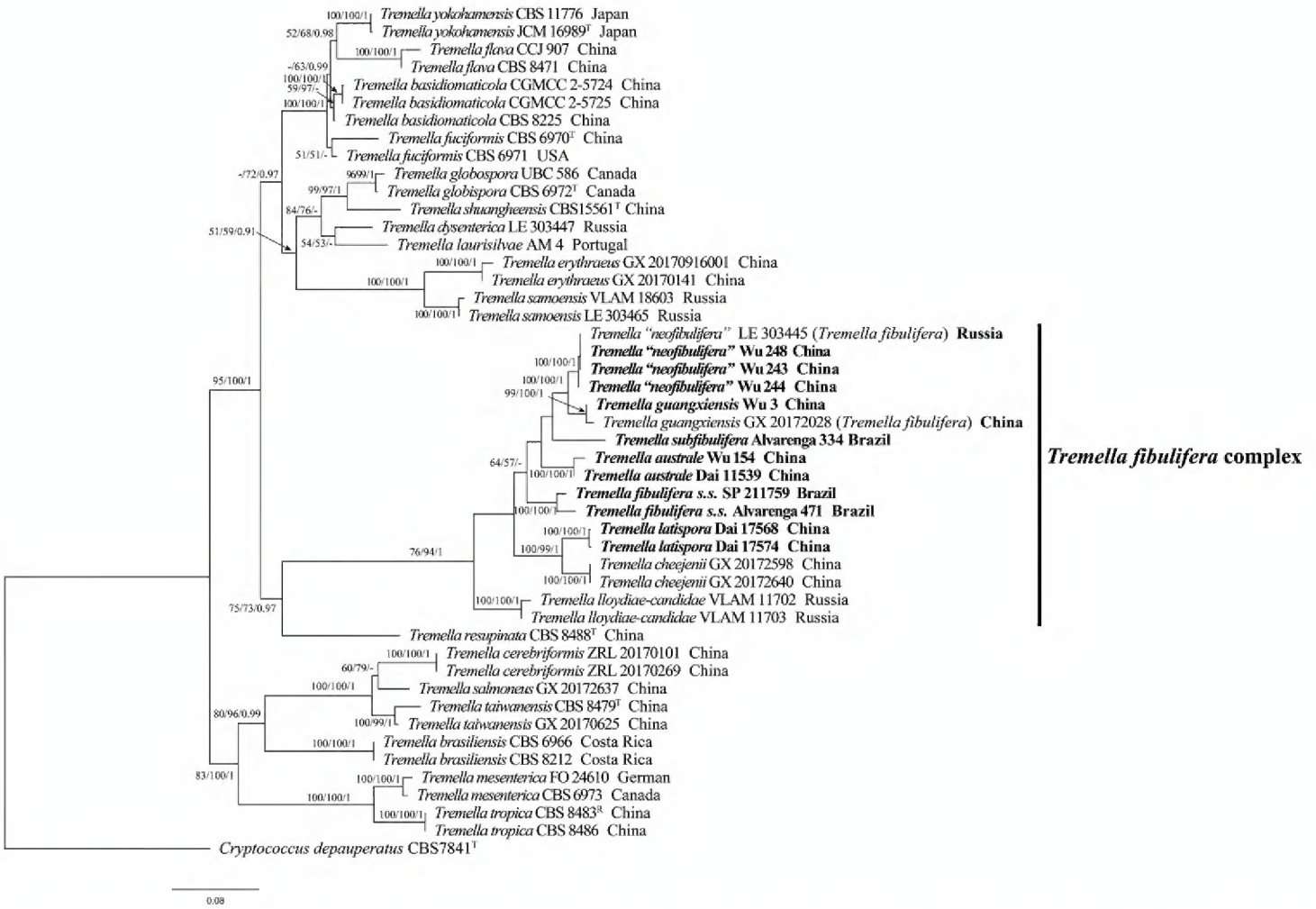
Tremella yokohamensis CBS 11776 Japan
Tremella yokohamensis JCM 16989T Japan
Tremella flava CCJ 907 China
Tremella flava CBS 8471 China
Tremella basidiomaticola CGMCC 2-5724 China
Tremella basidiomaticola CGMCC 2-5725 China
Tremella basidiomaticola CBS 8225 China
Tremella fuciformis CBS 6970T China
Tremella fuciformis CBS 6971 USA
Tremella globospora UBC 586 Canada
Tremella globispora CBS 6972T Canada
Tremella shuangheensis CBS15561T China
Tremella dysenterica LE 303447 Russia
Tremella laurisilvae AM 4 Portugal
Tremella erythraeus GX 20170916001 China
Tremella erythraeus GX 20170141 China
Tremella samoensis VLAM 18603 Russia
Tremella samoensis LE 303465 Russia
Tremella "neofibulifera" LE 303445 (Tremella fibulifera) Russia
Tremella "neofibulifera" Wu 248 China
Tremella "neofibulifera" Wu 243 China
Tremella "neofibulifera" Wu 244 China
Tremella guangxiensis Wu 3 China
Tremella guangxiensis GX 20172028 (Tremella fibulifera) China
Tremella subfibulifera Alvarenga 334 Brazil
Tremella australe Wu 154 China
Tremella australe Dai 11539 China
Tremella fibulifera s.s. SP 211759 Brazil
Tremella fibulifera s.s. Alvarenga 471 Brazil
Tremella latispora Dai 17568 China
Tremella latispora Dai 17574 China
Tremella cheejenii GX 20172598 China
Tremella cheejenii GX 20172640 China
Tremella lloydiae-candidae VLAM 11702 Russia
Tremella lloydiae-candidae VLAM 11703 Russia
Tremella fibulifera complex
Tremella resupinata CBS 8488T China
Tremella cerebriformis ZRL 20170101 China
Tremella cerebriformis ZRL 20170269 China
Tremella salmoneus GX 20172637 China
Tremella taiwanensis CBS 8479T China
Tremella taiwanensis GX 20170625 China
Tremella brasiliensis CBS 6966 Costa Rica
Tremella brasiliensis CBS 8212 Costa Rica
Tremella mesenterica FO 24610 German
Tremella mesenterica CBS 6973 Canada
Tremella tropica CBS 8483R China
Tremella tropica CBS 8486 China
Cryptococcus depauperatus CBS7841T
0.08

Figure 2. The Maximum likelihood tree showing phylogenetic relationship of species in *Tremella* s.s. based on the combined ITS + partial nLSU + mtSSU + TEF1 + RPB1 + RPB2 dataset. Bootstrap support values for MP and ML greater than 50% and BI greater than 0.95 are given at each node respectively. The samples used in this study are in bold.

471) from Brazil bearing the common feature of the complex formed a distinct lineage in our phylogenies (Figs 1, 2). Morphologically, the two specimens agree well with *T. fibulifera* except for the presence of conidia (Table 3). However, conidia are unstable in *T. fibulifera*. Möller (1895) described the anamorph of *T. fibulifera*, but the conidia were not observed when Bandoni and Oberwinkler (1983) re-described *T. fibulifera* based on the type designated by Möller (1895). Furthermore, *T. fibulifera* was originally described from Blumenau, Brazil, which is very close to the location of SP211759, Rondônia, Brazil. Therefore, we treat Alvarenga 471 and SP211759 as the representatives of *T. fibulifera* s.s. In addition, *T. fibulifera* s.s. are different from *T. subfibulifera* and *T. australe* by 8.51%, 9.87% sequence differences in the ITS sequences and 2.10%, 1.57% in the partial nLSU sequences respectively.

Tremella australe **F. Wu, L.F. Fan & Y.C. Dai, sp. nov.**
MycoBank No: 839825
Figs 3B, 5

Holotype. China Yunnan, Ruili, on fallen angiosperm branch, 23 April 2018, F. Wu 154 (BJFC028064).

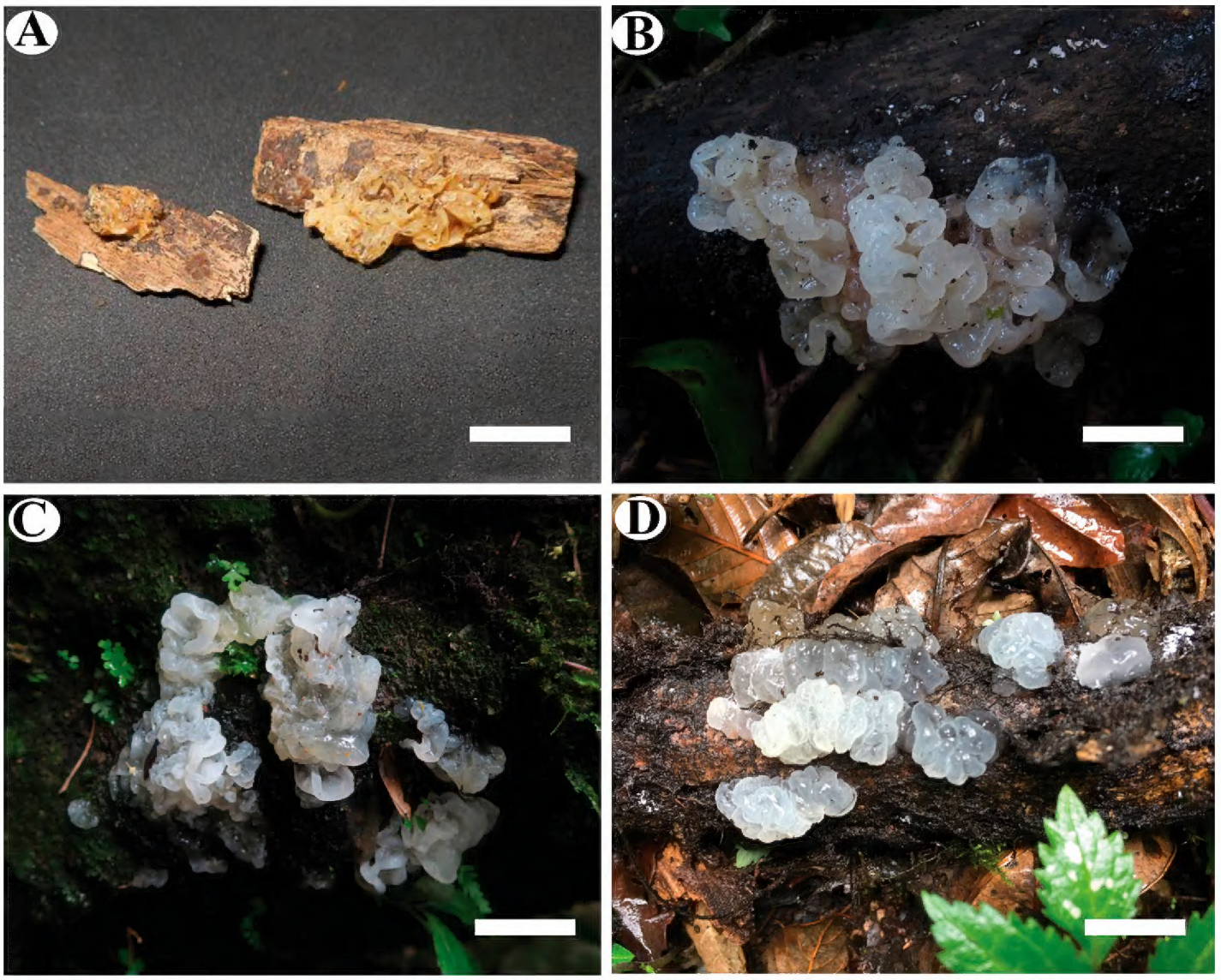

Figure 3. Basidioma **A** *Tremella fibulifera* (Alvarenga 471) **B** *T. australe* (Wu 154) **C** *T. guangxiensis* (Wu 3) **D** *T. latispora* (Dai 17568) **E** *T. "neofibulifera"* (Wu 244) **F** *T. subfibulifera* (Alvarenga 334). Scale bars: 1 cm (**A–F**).

Etymology. Refers to the distribution of this species in South Asia.

Basidioma. Sessile, when fresh soft gelatinous, creamy-white to beige, translucent, cerebriform, with thick and undulate lobes, up to 4.0 cm long, 2.0 cm broad and 2.0 cm high from base, distinctly shrinking into a film and becoming pale yellow when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, thin- to slightly thick-walled, 1.5–6.0 μm in diameter, branched, interwoven, with abundant clamp connections, clamp

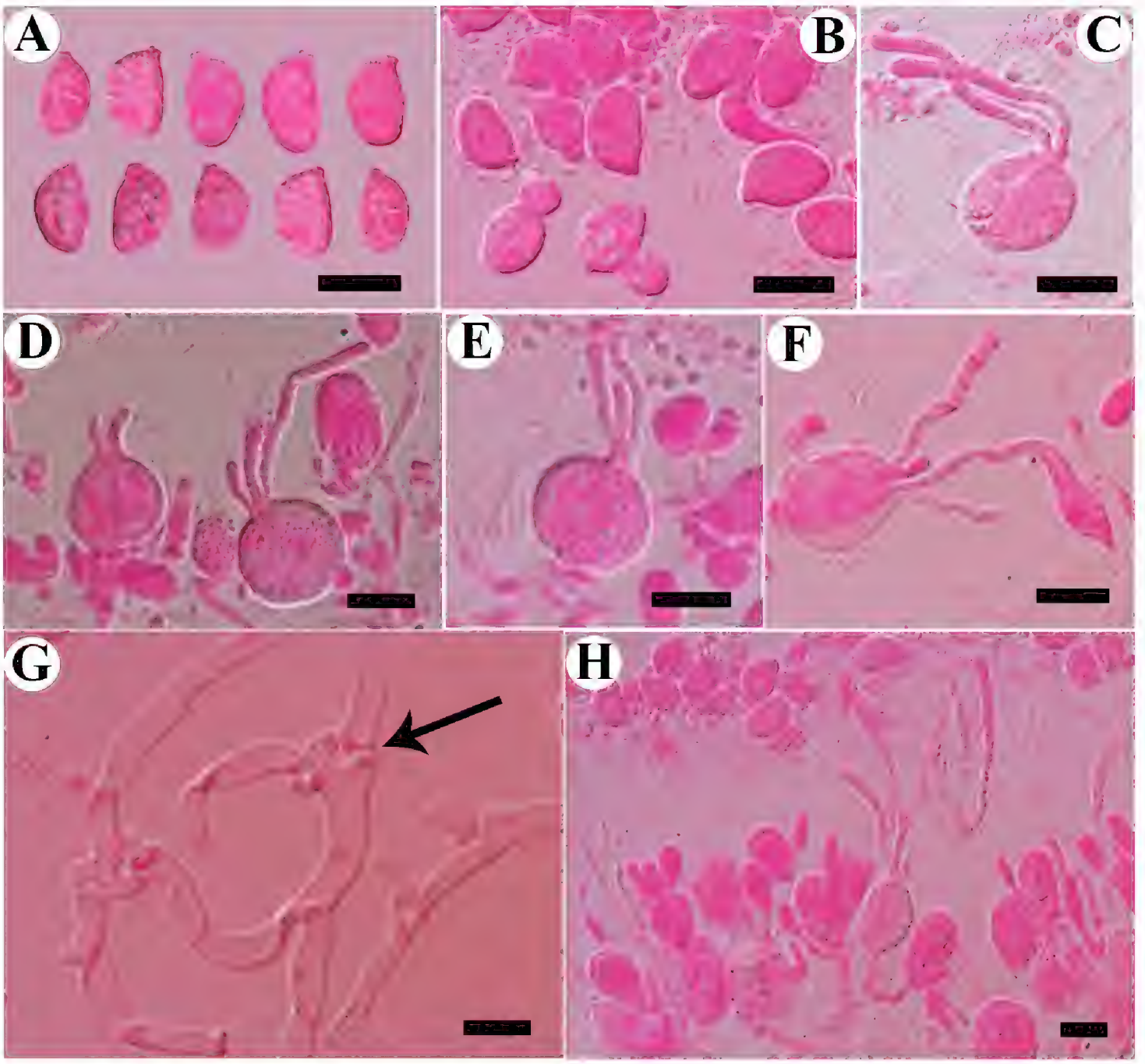

Figure 4. Microscopic structures of *Tremella fibulifera* s.s. (SP 211759) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C–F** basidia at different stages **G** hyphae with clamp connections and clamp complexes; **H** a section of hymenium. Scale bars: 10 μm (**A–H**).

complexes and anastomoses, slightly thick-walled hyphae usually present near to base of basidioma and sometimes swollen up to 8.5 μm; hyphidia hyaline, smooth, thin-walled, usually derived from the same hyphae with basidia; swollen cells, vesicles and haustoria absent; mature basidia thin-walled, globose to subglobose, with a basal clamp connection, 14.0–19.0 × 13.0–17.0(–18.0) μm, L = 16.3 μm, W = 15.8 μm, Q = 1.03 (n = 30/1), sometimes their width greater than length, usually longitudinally septate, 2–4-celled, with obvious oil drops; sterigmata up to 20 μm long, 1.0–2.5 in diameter, slightly protuberant at apex; probasidia thin-walled, globose to subglobose, mostly proliferating directly from basidial clamps; basidiospores hyaline, thin-walled, broadly ellipsoid to ellipsoid, apiculate, with oil drops, 8.0–10.0 × 6.0–8.0 μm, L = 8.6 μm, W = 7.3 μm, Q = 1.18–1.28 (n = 60/2), germinating by germ tubes or secondary spores; conidia absent.

Additional specimen examined. (***paratype***) China Taiwan, Yilan, Linmei Road, on fallen angiosperm branch, 20 June 2009, Y.C. Dai 11539 (BJFC007408).

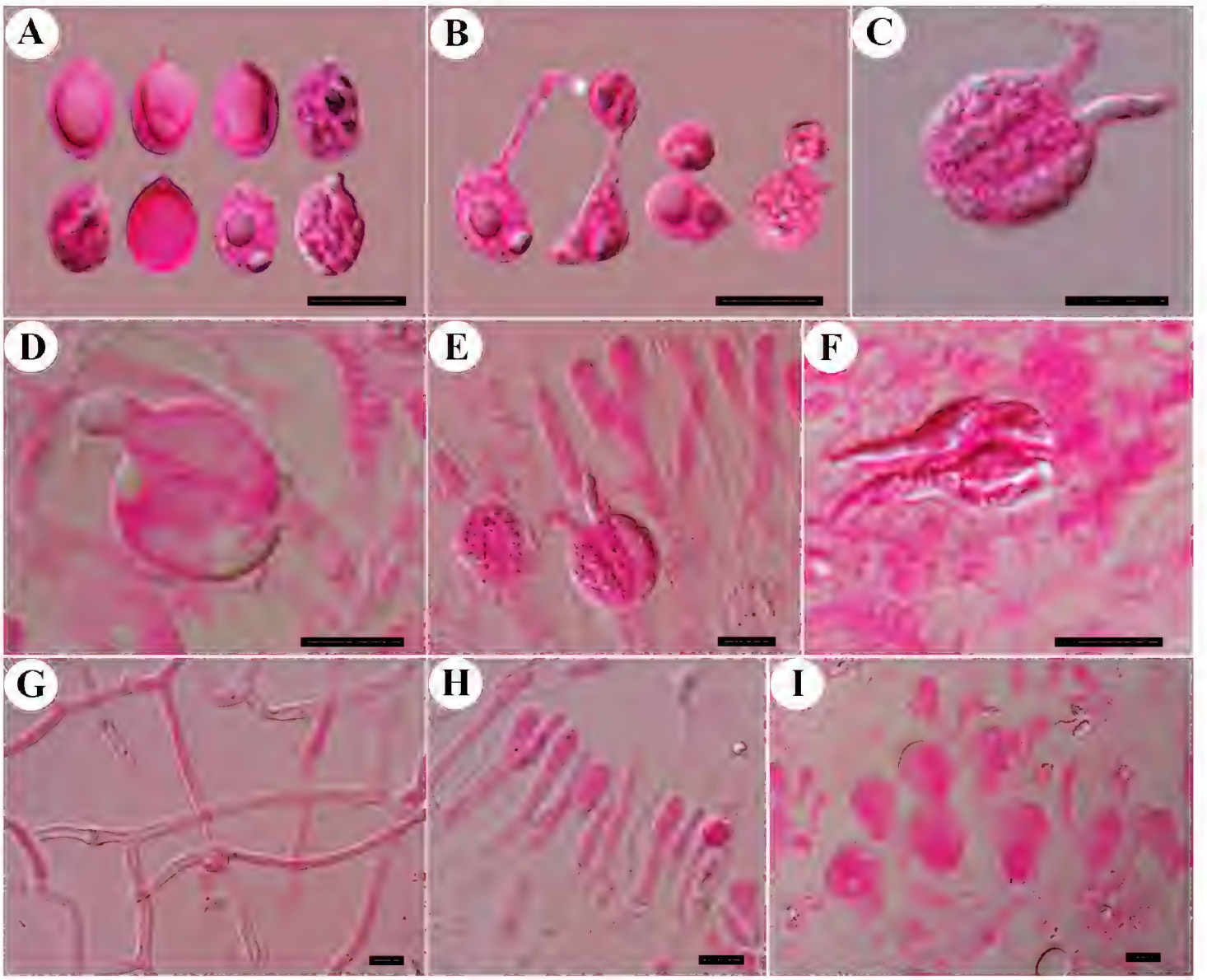

Figure 5. Microscopic structures of *Tremella australe* (Wu 154) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C–F** basidia at different stages **G** hyphae with clamp connections and clamp complexes **H** hyphidia **I** a section of hymenium. Scale bars: 10 μm (**A–I**).

Notes. *Tremella australe* formed an independent lineage with high support in our phylogenies (Figs 1, 2). The species is easily confused with *T. guangxiensis* by sharing whitish, translucent cerebriform basidioma and similar basidia and basidiospores, but *T. guangxiensis* has branched hyphidia and umbelliform conidiophores. Besides, *T. australe* are different from *T. subfibulifera*, *T. guangxiensis* and *T. "neofibulifera"* by 7.82%, 5.94% and 6.82% sequence differences in the ITS sequences and 2.13%, 3.43% and 1.25% in the partial nLSU sequences respectively.

Tremella guangxiensis **F. Wu, L.F. Fan & Y. C. Dai, sp. nov.**
MycoBank No: 839827
Figs. 3C, 6

Holotype. China. Guangxi, Jinxiu, Dayao Mountain, on angiosperm tree, 15 July 2017, F. Wu 3 (BJFC026009).

Etymology. Refers to the distribution of the species in Guangxi, China.

Basidioma. Sessile, when fresh soft gelatinous, milky to creamy-white, translucent, pustulate to irregularly cerebriform, with thick and undulate lobes, up to 4.0 cm long, 4.0 cm broad and 1.5 cm high from base, distinctly shrinking into a film and becoming lightly yellowish when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, thin- to slightly thick-walled, 2.0–6.0 μm in diameter, branched, interwoven, with abundant clamp connections, clamp complexes and anastomoses, slightly thick-walled hyphae usually present near to base of basidioma and sometimes swollen up to 9.0 μm; hyphidia hyaline, smooth, thin-walled, branched; swollen cells present, hyaline, smooth and various in the shape, sometimes slightly concave; vesicles and haustoria absent; mature basidia thin-walled, globose to subglobose, with a basal clamp connection, 14.0–17.0 × (13.6–)14.0–16.0(–17.0) μm, L = 15.9 μm, W = 14.8 μm, Q = 1.07 (n = 30/1), sometimes their

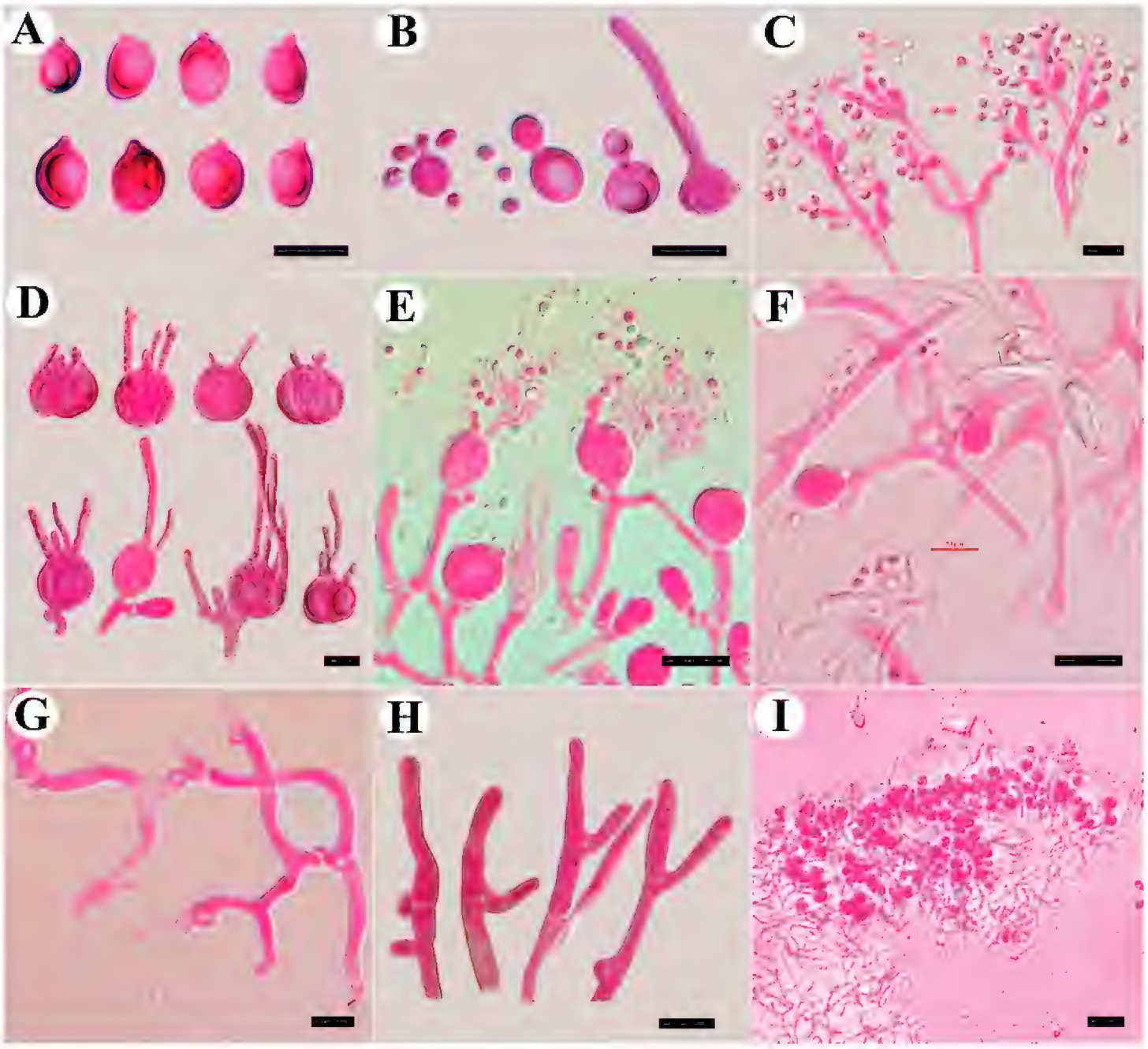

Figure 6. Microscopic structures of *Tremella guangxiensis* (Wu 3) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C** conidia and conidiophores **D** basidia at different stages **E, F** probasidia **G** hyphae with clamp connections and clamp complexes **H** hyphidia **I** a section of hymenium. Scale bars: 10 μm (**A–H**); 20 μm (**I**).

width greater than length, usually longitudinally septate, rarely obliquely septate, 2–4-celled, with obvious oil drops; sterigmata up to 60 μm long, 1.5–2.0 in diameter, slightly protuberant at apex; probasidia thin-walled, clavate to ellipsoid, proliferating from terminal hyphae; basidiospores hyaline, thin-walled, broadly ellipsoid to slightly ovoid, apiculate, with oil drops, (7.5–)8.0–9.5 × 6.0–7.5(–8.0) μm, L = 8.7 μm, W = 6.8 μm, Q = 1.28 (n = 30/1), germinating by germ tubes or secondary spores; conidia massively present, originating from umbelliform conidiophores, hyaline, thin-walled, ovoid to broadly ellipsoid or fusiform to cylindrical, 2.0–3.2 × 1.8–3.0 μm.

Notes. *Tremella guangxiensis* is closely related *T. "neofibulifera"* in our phylogenies (Figs 1, 2). The most distinctive characteristic of the species is branched hyphidia and umbelliform conidiophores, but *T. "neofibulifera"* has parallel hyphidia and lacks of conidia. In addition, *T. guangxiensis* are different from *T. australe* and *T. "neofibulifera"* by 6.35% and 5.09% sequence differences in the ITS sequences and 3.39% and 1.97% in the partial nLSU sequences respectively.

Tremella latispora **F. Wu, L.F. Fan & Y. C. Dai, sp. nov.**
MycoBank No: 839828
Figs 3E, 7

Holotype. China. Yunnan, Xinping, Shimenxia Park, on stump of *Lithocarpus*, 16 June 2017, Y.C. Dai 17574 (BJFC025106).

Etymology. Refers to the species having wide basidiospores.

Basidioma. Sessile, when fresh soft gelatinous, creamy-white to lvory, translucent, pustulate to irregularly cerebriform, with thick and undulate lobes, up to 4.0 cm long, 2.0 cm broad and 1.0 cm high from base, distinctly shrinking into a film and becoming whitish to pale yellow when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, thin- to thick-walled, 1.5–6.0 μm in diameter, branched, interwoven, with abundant clamp connections, clamp complexes and anastomoses, thick-walled hyphae usually present near to base of basidioma and sometimes swollen up to 7.5 μm; hyphidia hyaline, smooth, thin-walled, usually derived from the same hyphae with basidia; swollen cells, vesicles and haustoria absent; mature basidia thin-walled, globose to subglobose, with a basal clamp connection, 17.2–24.0(–27.0) × 17.0–23.0(–24.3) μm, L = 19.5 μm, W = 20.8 μm, Q = 0.94 (n = 30/1), commonly their width greater than length, usually longitudinally septate, occasionally obliquely septate, 2–4-celled, with obvious oil drops; sterigmata up to 60 μm long, 1.5–2.0 in diameter, slightly protuberant at apex; probasidia thin-walled, ellipsoid to subglobose, proliferating from terminal hyphae; basidiospores hyaline, thin-walled, globose to subglobose, apiculate, with oil drops, (9.0–)10.1–11.8(–12.0) × (9.6–)9.9–11.4(–11.7) μm, L = 11.0 μm, W = 10.7 μm, Q = 1.03 (n = 30/1), germination by germ tubes or secondary spores; conidia massively present, originating from umbelliform conidiophores, hyaline, thin-walled, ovoid to oblong or globose to subglobose, 2.8–3.6 × 1.8–3.0 μm.

Additional specimen examined. (***paratype***) China Yunnan, Xinping, Shimenxia Park, on stump of *Lithocarpus*, 16 June 2017, Y.C. Dai 17568 (BJFC025100).

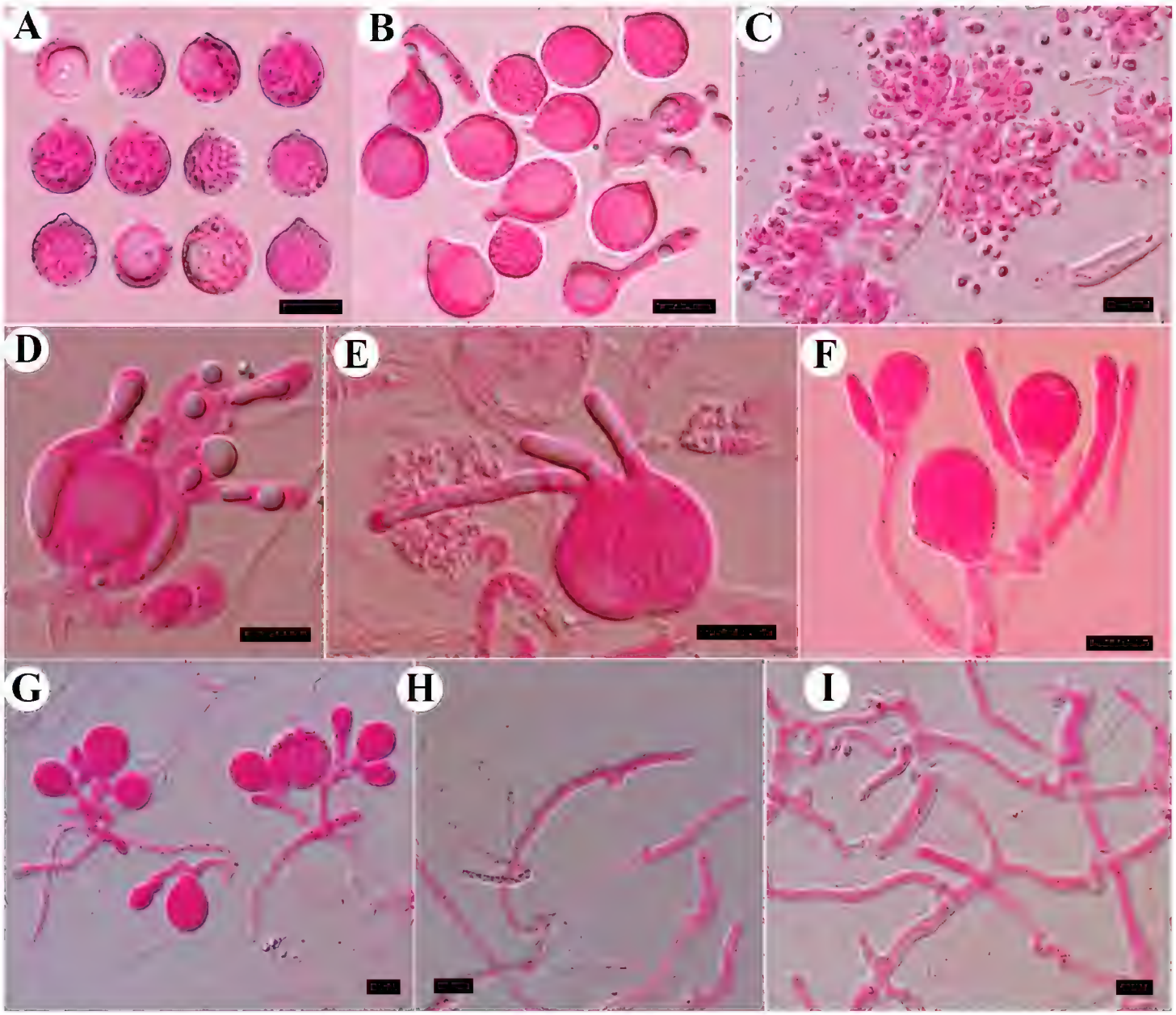

Figure 7. Microscopic structures of *Tremella latispora* (Dai 17568) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C** conidia and conidiophores **D, E** basidia **F, G** probasidia **H, I** hyphae with clamp connections and clamp complexes. Scale bars: 10 μm (**A–I**).

Notes. Phylogenetically, *Tremella latispora* formed a distinct lineage closely related to *T. cheejenii* (Figs 1, 2). Morphologically, the species has significantly larger basidia and basidiospores than *T. cheejenii* or other similar species (Table 3), and it has globose to subglobose basidiospores rather than more or less ellipsoid basidiospores in other species. And *T. latispora* are different from *T. cheejenii* and *T. fibulifera* s.s. by 4.63% and 5.09% sequence differences in the ITS sequences and 3.39% and 2.95% in the partial nLSU sequences respectively.

***Tremella "neofibulifera"* Kobayasi, Scientific Report, Tokyo Bunrika Daigaku, Section 4: 15 (1939)**
Figs 3D, 8

Basidioma. Sessile, when fresh soft gelatinous, creamy-white to pale yellowish, irregularly cerebriform or slightly foliose, with undulate lobes, up to 4.5 cm long, 2.0 cm

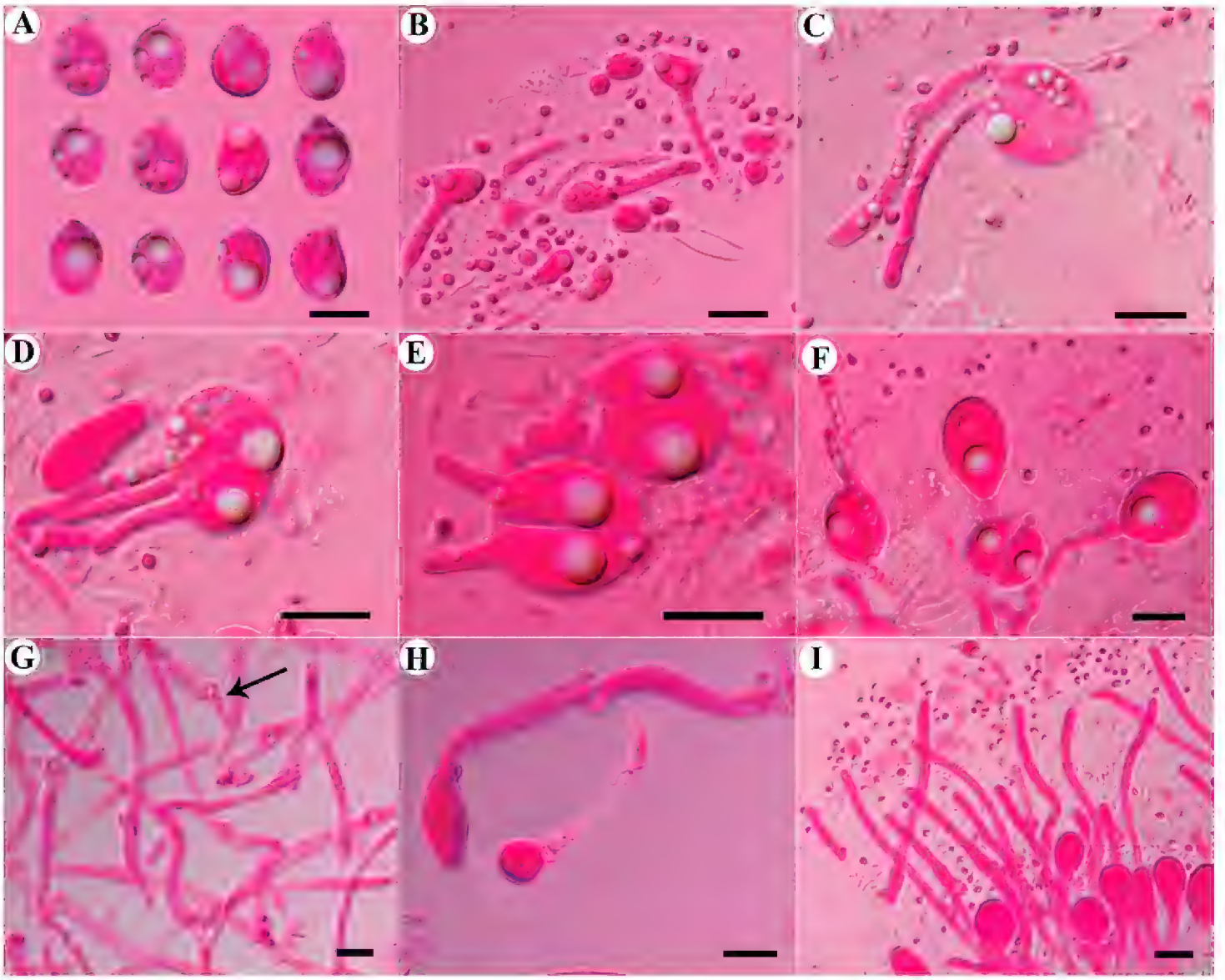

Figure 8. Microscopic structures of *Tremella "neofibulifera"* (Wu 248) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C–E** basidia at different stages **F** probasidia **G** hyphae with clamp connections and clamp complexes **H** vesicles **I** parallel hyphidia in hymenium. Scale bars: 10 μm (**A–I**).

broad and 2.5 cm high from base, becoming firmly gelatinous and invisible yellowish when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth thin- to slightly thick-walled, 2.0–6.0 μm in diameter, branched, interwoven, with abundant clamp connections, clamp complexes and anastomoses, slightly thick-walled hyphae usually present near to base of basidioma, sometimes swollen up to 8.5 μm; hyphidia hyaline, smooth, thin-walled, arranged in cluster, usually parallel; vesicles infrequent, thick-walled; swollen cells and haustoria absent; mature basidia thin-walled, ovoid to subglobose, with a basal clamp connection, 14.0–16.0 × 13.0–17.0 μm, L = 14.9 μm, W = 14.8 μm, Q = 1.01 (n = 30/1), sometimes their width greater than length, usually longitudinally septate, rarely obliquely septate, 2–4-celled, with obvious oil drops; sterigmata up to 70 μm long, 1.5–2.0 in diameter, slightly protuberant at apex; probasidia thin-walled, ellipsoid to subglobose, usually proliferating from terminal hyphae; basidiospores hyaline, thin-walled, ellipsoid to broadly ellipsoid, apiculate, with oil drops, 8.0–10.0 × 6.0–8.0 μm, L = 8.9 μm, W = 6.5 μm, Q = 1.37 (n = 30/1), germination by germ tubes or secondary spores; conidia absent.

Specimens examined. China Jilin, Helong, Quanshuidong Forest Farm, on stump of *Quercus*, 15 July 2017, F. Wu 243 (BJFC031046); F. Wu 244 (BJFC031047); F. Wu 248 (BJFC031051).

Notes. Three specimens listed above from Northeast China together with LE303445 from Far East of Russia formed a distinct lineage closely related to *T. guangxiensis* in our phylogenies (Figs 1, 2). *T. neofibulifera* was originally described from Japan (Kobayasi 1939), and our studied East Asian samples have similar morphology to *T. neofibulifera* except bigger basidiospores (Table 3). We fail to loan the type of *T. neofibulifera*, and for the time being we treat our studied East Asia samples as *T. "neofibulifera"*. The current *T. "neofibulifera"* differs from other similar species of the *Tremella fibulifera* complex by the parallel hyphidia and the presence of vesicles. In addition, *T. "neofibulifera"* are different from *T. guangxiensis*, *T. australe*, *T. subfibulifera* and *T. fibulifera* s.s. by 3.15%, 5.25%, 7.14%, and 8.19% sequence differences in the ITS sequences and 2.04%, 1.32%, 3.18%, and 2.41% in the partial nLSU sequences respectively.

Tremella subfibulifera **Alvarenga, F. Wu, L.F. Fan & Y.C. Dai, sp. nov.**
MycoBank No: 839829
Figs 3F, 9

Holotype. Brazil. Pernambuco, Recife, Jardim Botânico do Recife, on angiosperm wood, 17 June 2016, R. L. M. Alvarenga 334 (URM).

Etymology. Refers to the species being similar to *Tremella fibulifera*.

Basidioma. Sessile, when fresh gelatinous, pale white, foliose to irregularly cerebriform, with undulate lobes, up to 3.0 cm long, 2.0 cm broad and 1.0 cm high from base, becoming firmly gelatinous and pale yellowish when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, slightly thick-walled, 2.0–4.0 µm in diameter, branched, interwoven, with abundant clamp connections, clamp complexes and anastomoses; hyphidia, swollen cells, vesicles and haustoria absent; mature basidia thin-walled, subglobose to broadly ellipsoid, with a basal clamp connection, (14.0–)14.4–20.3(–21.0) × (9.0–)12.8–16.3(–17.8) µm, L = 17.63 µm, W = 15.05 µm, Q = 1.17 (n = 30/1), sometimes their width greater than length, usually longitudinally or obliquely septate, 2–4-celled, with obvious oil drops; mature sterigmata often collapsed, juvenile sterigmata up to 15.0 µm long, 2.0–4.0 µm in diameter, slightly protuberant at apex; probasidia thin-walled, clavate to ellipsoid, guttulate, proliferating from terminal hyphae; basidiospores hyaline, thin-walled, ellipsoid apiculate, with oil drops, (5.0–)5.4–9.8(–10.0) × (4.0–)4.2–6.0(–6.4) µm, L = 8.0 µm, W = 5.3 µm, Q = 1.50 (n = 30/1); conidia massively present, originating from umbelliform conidiophores, hyaline, thin-walled, variously shaped, ellipsoid, fusiform to cylindrical, 2.0–3.0 × 0.5–1.0 µm.

Notes. *Tremella subfibulifera* nested in the clade of the *T. fibulifera* complex, and formed an independent lineage. It resembles *T. fibulifera* s.s., but *T. fibulifera* s.s. has larger basidiospores (7.0–10.0 × 6.0–7.0 µm vs. 5.4–9.8 × 4.2–6.0 µm) and the pres-

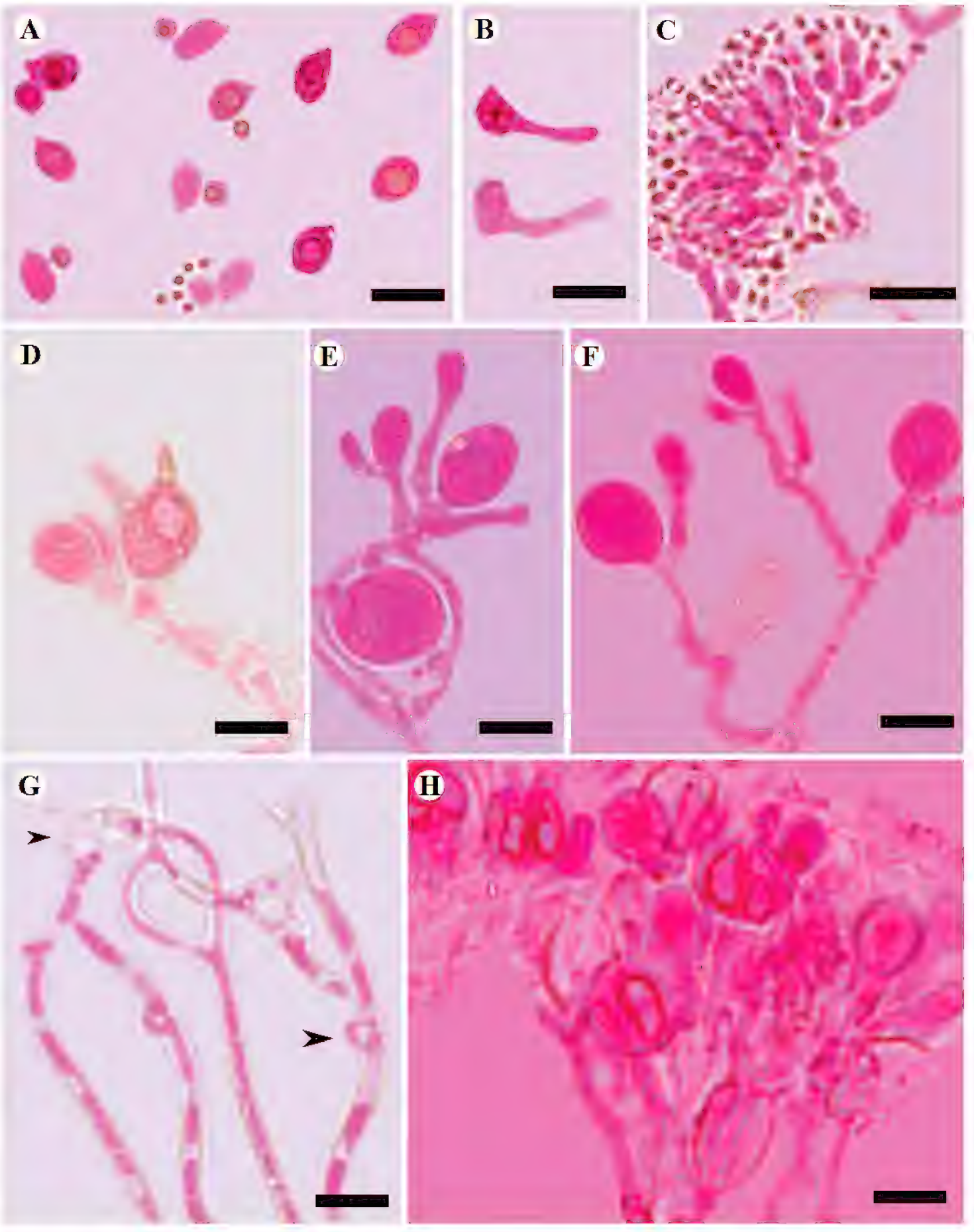

Figure 9. Microscopic structures of *Tremella subfibulifera* (Alvarenga 334) **A** basidiospores and secondary spores **B** germination tubes of basidiospores **C** conidia and conidiophores **D–F** basidia and probasidia **G** hyphae with clamp connections and clamp complexes **H** a section of hymenium. Scale bars: 10 μm (**A–H**).

ence of branched hyphidia (Table 3). In addition, *T. subfibulifera* are different from *T. australe* and *T. fibulifera* s.s. by 6.19% and 7.85% sequence differences in the ITS sequences and 2.23% and 2.10% in the partial nLSU sequences respectively.

Discussion

Tremella fibulifera was originally described from Blumenau of Brazil (Möller 1895); later two similar species, *T. olens* and *T. neofibulifera*, were respectively described from Tasmania of Australia and Simotuke of Japan (Hooker 1860; Kobayasi 1939). The Russian Far East specimen LE303445 was identified as *T. fibulifera* by Malysheva et al. (2015). Our results demonstrated the Northeastern Chinese specimens and Russian Far East specimen formed an independent lineage, and this lineage is distantly related to the lineage formed by two Brazilian specimens, SP 211759 and Alvarenga 471 (Figs 1, 2). The location of SP 211759 is near to the type locality of *T. fibulifera*. So, we treat SP 211759 and Alvarenga 471 as representatives of *T. fibulifera* s.s. Molecular data are not available from type or type locality specimens of *T. neofibulifera*. Neither is its type re-examined, but the Northeastern Chinese specimens have more or less similar morphology as the description of *T. neofibulifera*, so we temporarily treat Northeast Chinese specimens and Russian Far East specimen as *T. "neofibulifera"*.

The Southern Chinese specimen GX20172028 was also identified as *Tremella fibulifera* by Zhao et al. (2019), but it clustered with another Southern Chinese specimen Wu 3 into a distinct lineage which is closely related to *T. "neofibulifera"* (Figs 1, 2). *T. guangxiensis* is different from *T. "neofibulifera"* by 5.09% sequence differences in the ITS sequences and 1.97% in the partial nLSU sequences respectively. In addition, the Southern Chinese specimens have translucent basidioma, branched hyphidia and umbelliform conidiophores, and they are readily distinguished from *T. "neofibulifera"*. So, these two specimens are identified as a new species *T. guangxiensis*.

Seven species, *Tremella fibulifera*, *T. olens*, *T. "neofibulifera"*, *T. guangxiensis*, *T. australe*, *T. latispora* and *T. subfibulifera* have cerebriform whitish basidioma and abundant clamp complexes, and they nested in the same clade. So, we treat these seven species as members of the *T. fibulifera* complex.

Tremella lloydiae-candidae Wojewoda and *T. cheejenii* Xin Zhan Liu & F.Y. Bai also have whitish basidioma and similar micro-morphology with *T. fibulifera*, but clamp complexes were not observed (Malysheva et al. 2015; Zhao et a. 2019), and we did not examine their types. Because these two species are nested in the same clade as other species of the *T. fibulifera* complex with robust support in our phylogenies (Figs 1, 2), we treat them as members of the *T. fibulifera* complex, too.

Currently, more than 30 morphological characteristics are applied for identification species of *Tremella* s.s. (Chen 1998; Zhao et al. 2019), and some features including basidioma color and basidia shape are variable at different stages. The shape and size of basidiospores are relatively stable characteristics for each species, but they are very similar among some species in the *T. fibulifera* complex; that is why several taxa were previously treated as *T. fibulifera* s.l. (Malysheva et al. 2015; Zhao et al. 2019). Consequently, combined morphology and molecular evidence are essential to distinguish species within the complex, and ITS + partial nLSU dataset are selected for species delimitation.

Key to the whitish species in *Tremella* s. s.

1	Basidiospores > 10 μm long	**2**
–	Basidiospores < 10 μm long	**5**
2	Basidioma resupinate	***T. resupinata***
–	Basidioma pustulate to irregularly cerebriform or foliose	**3**
3	Basidiospores > 17 μm long	***T. cerebriformis***
–	Basidiospores < 17 μm long	**4**
4	Basidiospores > 12 μm long	***T. olens***
–	Basidiospores < 12 μm long	***T. latispora***
5	Basidia with stalks	**6**
–	Basidia without stalks	**8**
6	Basidia < 13 μm wide	***T. yakohamensis***
–	Basidia > 13 μm wide	7
7	Basidiospores mostly broader than long	***T. globispora***
–	Basidiospores mostly longer than broad	***T. cheejenii***
8	Basidia with sterigmata shorter than 35 μm	**9**
–	Basidia with sterigmata longer than 35 μm	**11**
9	Basidiospores < 6 μm wide	***T. subfibulifera***
–	Basidiospores > 6 μm wide	**10**
10	Hyphae with clamp complexes and anastomoses	***T. australe***
–	Hyphae without clamp complexes and anastomoses	***T. lloydiae-candidae***
11	Basidioma filamentous lobes, conjunctive as a ball	***T. hainanensis***
–	Basidioma pustulate to irregularly cerebriform or foliose	**12**
12	Basidiospores < 6 μm wide	***T. fuciformis***
–	Basidiospores > 6 μm wide	13
13	Hyphidia parallel; conidia absent	***T. "neofibulifera"***
–	Hyphidia branched; conidia present	**14**
14	Basidioma pustulate to irregularly cerebriform; basidia with sterigmata up to 60 μm; conidia originating from umbelliform conidiophores	***T. guangxiensis***
–	Basidioma lobed to irregularly cerebriform; basidia with sterigmata up to 100 μm; conidia not originating from umbelliform conidiophores	***T. fibulifera*** **s.s.**

Acknowledgements

We sincerely thank Drs. Li-Wei Zhou (Shenyang, China), Jun-Zhi Qiu (Fujian, China), Bao-Kai Cui (Beijing, China), Shuang-Hui He (Beijing, China), Jun-Liang Zhou (Yunnan, China), Hai-Xia Ma (Hainan, China) and Adriana de Mello Gugliotta (Brazil) for collecting and providing specimens. The research was financed by the National Natural Science Foundation of China (Project Nos. 31701978, U1802231), CNPq (PQ 1D, 307601/2015-3, 302941/2019-3; ICMBio 421241/2017-9) and FACEPE (APQ 0003-2.03/18).

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